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A β 42 assembles into specific β -barrel pore-forming oligomers in membrane-mimicking environments

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The formation of amyloid- β peptide (A β) oligomers at the cellular membrane is considered to be a crucial process underlying neurotoxicity in Alzheimer's disease (AD). Therefore, it is critical to characterize the oligomers that form within a membrane environment. To contribute to this characterization, we have applied strategies widely used to examine the structure of membrane proteins to study the two major A β variants, A β 40 and A β 42. Accordingly, various types of detergent micelles were extensively screened to identify one that preserved the properties of A β in lipid environments—namely the formation of oligomers that function as pores. Remarkably, under the optimized detergent micelle conditions, A β 40 and A β 42 showed different behavior. A β 40 aggregated into amyloid fibrils, whereas A β 42 assembled into oligomers that inserted into lipid bilayers as well-defined pores and adopted a specific structure with characteristics of a β -barrel arrangement that we named β -barrel pore-forming A β 42 oligomers (β PFOs_{A β 42}). Because A β 42, relative to A β 40, has a more prominent role in AD, the higher propensity of A β 42 to form β PFOs constitutes an indication of their relevance in AD. Moreover, because β PFOs_{A β 42} adopt a specific structure, this property offers an unprecedented opportunity for testing a hypothesis regarding the involvement of β PFOs and, more generally, membrane-associated A β oligomers in AD.

Alzheimer's disease | amyloid- β peptide | membrane pore | oligomer

One of the main pathological features of Alzheimer's disease (AD) is the extracellular accumulation of the amyloid-beta peptide (A β) as fibrillar amyloid plaques (1). A β is obtained from the transmembrane amyloid precursor protein (APP) by consecutive action of the enzymes β -secretase and γ -secretase. The cleavage of γ -secretase occurs sequentially, giving rise to amphipathic A β peptides of lengths ranging from 38 to 43 residues (A β 38 to A β 43) (2). Because amyloid plaques are detected extracellularly, it is generally considered that, after APP processing, A β variants are fully released into the extracellular media. It is for this reason that researchers have focused on the study of A β in a solution environment (3–5). However, several of the most likely mechanisms of A β neurotoxicity are associated with the cell membrane, including interactions with membrane receptors (6), induction of membrane bilayer disorder (7), and generation of amyloid pores or channel-like structures (8–12). Therefore, apart from studying A β in solution, it is crucial to examine this peptide in a membrane environment.

As in all membrane-associated research, it is of paramount importance to use a suitable biomimetic membrane environment. In this context, previous studies devoted to A β in a membrane environment have used either liposomes (8–12) or detergent micelles (13–15). The study of A β reconstituted in liposomes has been mainly through electrical recording using planar lipid bilayers. Pioneering research by Arispe et al. revealed that this peptide forms pores across phospholipid bilayer membranes and that these pores show spontaneous transitions between defined levels of conductance (8, 9). This work led to the “amyloid pore hypothesis,” which proposes the formation of A β pores at the membrane as a key process in the neurotoxicity observed in AD. Subsequent

research by other groups led to somewhat conflicting results due to the diversity of the A β pores reported (10–12). Such diversity has prevented the identification of specific features that define A β pore structure and conductivity properties, thus preventing confirmation of the amyloid pore hypothesis.

Detergent micelles are commonly used to solubilize membrane proteins for structural research. Indeed, their small size—compared with that of other biomimetic membrane environments—enables the application of well-established solution NMR techniques, thus allowing the obtention of high-resolution structural information for the membrane protein under study (16). However, unlike lipid bilayers, detergent micelles are spheroid and vary in shape and size depending on the chemical structure of the detergent. Therefore, when using detergents to study membrane protein structure and function, several types of detergent micelles must be extensively screened to find one that preserves the native structure and function of the membrane protein under study (16, 17). Such screening has not been performed when studying A β in the presence of detergent micelles. Instead, multiple detergents and conditions have been used, leading to diverse and contradictory results ranging from A β being monomeric and adopting an α -helical structure (13) to A β forming oligomers rich in β -sheet structure (14, 15). Moreover, the links between the A β species formed in the presence of detergent micelles and those formed in lipid environments has not been established.

In the present paper, we contribute to establishing such a link. We report on micelle conditions to prepare specific A β oligomers with the same function as that observed for A β oligomers formed in a lipid environment—namely pore formation in lipid

Significance

Numerous reports indicate that amyloid- β peptide (A β) oligomers, considered the pathogenic molecular form of A β in Alzheimer's disease (AD), exert their neurotoxicity within the membrane. Therefore, it is critical to characterize them in such an environment. Here, we worked with two major A β variants and handled them as if they were membrane proteins. By doing so, we found that the A β variant most strongly linked to AD assembled into stable A β oligomers that adopted a specific structure and incorporated into membranes as pores, a feature linked to neurotoxicity. Having access to pore-forming A β oligomers with such a specific structure offers unique opportunities to fully characterize them and establish their involvement in AD.

Author contributions: M.S.-B., M.N.-P., M.B., G.M., and N.C. designed research; M.S.-B., M.N.-P., and M.B. performed research; M.G. contributed new reagents/analytic tools; M.S.-B., M.N.-P., M.B., G.M., and N.C. analyzed data; and N.C. wrote the paper.

The authors declare no conflict of interest.

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population of A β 42 oligomers (*SI Appendix, Fig. S2F*). Indeed, calibration of the SEC column with globular protein standards indicated that the A β 42 oligomer–micelle complex had a mass of approximately 60 kDa (*SI Appendix, Fig. S3*).

Having observed such remarkable evolution for the A β 42 oligomer samples formed under DPC micelle conditions (Fig. 1 and *SI Appendix*, Fig. S2F), we considered it appropriate to further characterize the behavior of A β 40 under the same conditions (Fig. 1). A β 40 samples reconstituted under high DPC micelle conditions eluted like the monomeric SDS control before and after incubation, thereby indicating that A β 40 remained monomeric throughout the incubation period. In contrast, A β 40 samples reconstituted under low DPC micelle conditions eluted like the monomeric SDS control before the incubation period, but hardly any signal was detected in SEC after completion of the incubation. These results reveal that initially A β 40 was monomeric and that it assembled into large aggregates with time. To learn more about the morphology of these aggregates, we used transmission electron microscopy (TEM), which showed the presence of abundant amyloid fibrils (Fig. 1B). In summary, under high DPC micelle conditions and after 24 h incubation at 37 °C, A β 40 remained monomeric and A β 42 assembled into size homogeneous oligomers (Fig. 1). In contrast, under low DPC micelle conditions and after 24 h incubation at 37 °C, A β 40 aggregated into amyloid fibrils whereas A β 42 continued to assemble into size homogeneous oligomers (Fig. 1). Because the latter condition mimicked two possible extreme scenarios for A β in the brain of AD patients—evolution into amyloid fibrils, as observed for A β 40, and assembly

Figure 1 Size exclusion chromatography (SEC) and electron microscopy (EM) analysis of A β 40 and A β 42 aggregates. Panel A shows SEC chromatograms for A β 40 and A β 42 at 0 h and 24 h. The y-axis is mAU at 220 nm (0 to 300) and the x-axis is elution volume (mL) (20 to 40). Peaks are labeled M (monomer) and O (oligomer). Panel B shows EM images of the aggregates at 0 h and 24 h. Scale bars are present in the EM images.

Fig. 1. Evolution of A β 40 and A β 42 samples prepared under low and high DPC micelle conditions. Schematics of four micelles and one micelle, shown in green, represent high and low micelle conditions, respectively. Samples were prepared at 150 μ M A β under low and high DPC micelles at pH 7.4 and analyzed immediately after being reconstituted ($t = 0$ h) and after 24 h incubation at 37 $^{\circ}$ C. SEC chromatograms (A) and electron micrographs (B) at the indicated times. In SEC chromatograms, the orange and purple lines correspond to the elution volumes of the monomeric A β 40 control (*SI Appendix, Fig. S1A*) and the A β oligomers formed under the studied conditions, respectively. The width of the purple line represents the size distribution of the A β oligomers formed under each of the studied conditions. (Scale bars, 100 nm.)

into membrane-associated oligomers, as observed for A β 42—we chose it as the conditions to pursue further studies.

The A β 42 Oligomers Stabilized by DPC Micelles Insert into Lipid Bilayers as Well-Defined Pores. Next, we continued to apply established strategies in the field of membrane proteins and tested whether the A β 42 oligomers formed under the chosen conditions preserved the same pore functionality as observed for A β oligomers formed in lipid environments (8–12). To this end, we used electrical recordings with planar lipid bilayers. Samples corresponding to A β 40 prepared under low DPC micelle conditions, to A β 42 and A β 40 monomers, and to empty micelles were also analyzed. Neither the A β 40 nor the empty micelle samples showed any activity in lipid bilayers. The addition of A β 42 monomers to the cis side of a planar lipid bilayer induced fast, transient, and heterogeneous ionic current events from approximately –10 to approximately –40 pA at –200 mV (SI Appendix, Fig. S4A). This “spiky” behavior has been reported for A β and attributed to the formation of a highly heterogeneous population of A β pores (10, 12). The addition of A β 42 oligomers prepared under low DPC micelle conditions often led to transient disruptions of bilayer conductance (SI Appendix, Fig. S4B), similar to those observed when A β 42 monomers were allowed to interact with the lipid bilayer (SI Appendix, Fig. S4A). However, 5–15 min after the addition of the A β 42 oligomers to the chamber, step-wise changes in bilayer conductance were observed (Fig. 2A), behavior typical of the incorporation of individual proteins that form nanopores in membrane bilayers (21). As these oligomers induced various types of nanopore-like behavior, we grouped the responses into three classes, denoted type 1, 2, and 3 on the basis of the signal observed (Fig. 2B–D). Type 1, observed in approximately 17% of the experiments ($n = 105$), was characterized by fast and noisy transitions with undefined open pore conductance values (current levels ranging from –40 to –100 pA at –100 mV) (Fig. 2B). Type 2, observed in approximately 48% of the experiments, showed a reasonably well-defined open pore conductance (current level approximately –20 pA at –100 mV) accompanied by significant, rapidly fluctuating noise [root-mean-square (rms) noise $> \sim 4$ pA at –100 mV applying a 2-kHz Bessel filtering] (Fig. 2C). Finally, type 3, which was observed in 35% of the experiments, indicated the presence of a well-defined open pore with no current fluctuations (current level –20 pA at –100 mV) (Fig. 2D). These nanopore-like currents typically appeared to be irreversible, although opening and closing events were occasionally observed. Type 2 and type 3 conductance showed an average open pore current of -19.2 ± 3.5 pA at –100 mV (SI Appendix, Fig. S4C and D). Moreover, assuming that the internal surface of the A β 42 oligomeric pores does not affect their conductivity and considering that the pore length is 3 nm, which corresponds to the hydrophobic length of a lipid bilayer, type 2 and 3 pores are consistent with a cylinder with an inner diameter of approximately 0.7 nm (22). All together, these results revealed that A β 42 oligomers prepared under low DPC micelle conditions

have the capacity to form pores in lipid bilayers, the same as A β in lipid environments (8–12).

The Pore-Forming A β 42 Oligomers Adopt a Specific Structure with Characteristics of a β -Barrel Arrangement. Having confirmed the pore-forming capacity of the A β 42 oligomers, we proceeded with their structural characterization. Although initially we prepared the oligomer sample at pH 7.4, it was found to have the same structure while being more stable when prepared at pH 9.0 (SI Appendix, Fig. S5). Therefore, we conducted all structural characterization at pH 9.0. Initially, we characterized the A β 42 oligomers by using carbon-13 incorporation into the methyl group of the methionine side chain (Met-[$^{13}\text{CH}_3$]). These methyl groups are highly dynamic and thus have longer relaxation times than those of most hydrogen and carbon atoms in the protein. Moreover, because the sequence of A β 42 contains a single methionine at residue 35 (Fig. 3A), Met 35 -[$^{13}\text{CH}_3$] A β 42 labeling offers the additional advantage of spectral simplification. ^1H - ^{13}C HMQC spectra of the Met 35 -[$^{13}\text{CH}_3$] A β 42 oligomer sample led to the observation of two sharp and dispersed peaks (Fig. 3B), indicating that the Met-35 side chain perceives two well-defined structural environments. To obtain further information on the number of environments at different sites of the A β 42 peptide sequence, we measured the ^1H - ^{15}N HSQC spectra of the ^2H , ^{15}N A β 42 oligomer sample and focused on the region of the spectra characteristic of the side-chain amides of Gln and Asn residues. Residues 15 and 27 in the A β 42 sequence are Gln and Asn, respectively (Fig. 3A). Each side chain amide of Asn and Gln results in two peaks with identical ^{15}N but different ^1H chemical shifts. We observed eight peaks, consistent with Asn and Gln chains also perceiving two well-defined environments (Fig. 3C).

Next, to learn about the average secondary structure adopted by the A β 42 oligomer, we analyzed it by circular dichroism (CD). CD spectra revealed a minimum at 218 nm, indicating that the oligomer adopted a β -sheet structure (Fig. 3D). Further evidence of this type of secondary structure was obtained by analyzing the fingerprint region of ^1H - ^{15}N TROSY-HSQC spectrum of the ^2H , ^{15}N A β 42 oligomer sample (Fig. 3E). The spectrum showed that signals appeared in two differentiated regions—a set of approximately 27 peaks, clustered in the random coil region and a set of 37 downfield-shifted, the latter characteristic of a protein assembly dominated by β -sheet secondary structure. A β 42 has 42 residues, and NMR experiments using Gln-15, Asn-27, and Met-35 side chains as probes indicated that this peptide adopted two distinct environments (Fig. 3B and C). Therefore, we expected to detect 82 peaks in the ^1H - ^{15}N TROSY-HSQC, but only detected 64. This result may be explained because of peak overlap and the fact that at pH 9.0, backbone amides of residues comprising disordered regions of the oligomer undergo fast exchange with the solvent, a process that results in signal loss. To further characterize the two distinct regions of the ^1H - ^{15}N TROSY-HSQC spectrum, we carried out hydrogen/deuterium exchange (HDX) experiments, which revealed that peaks clustered in the random coil region

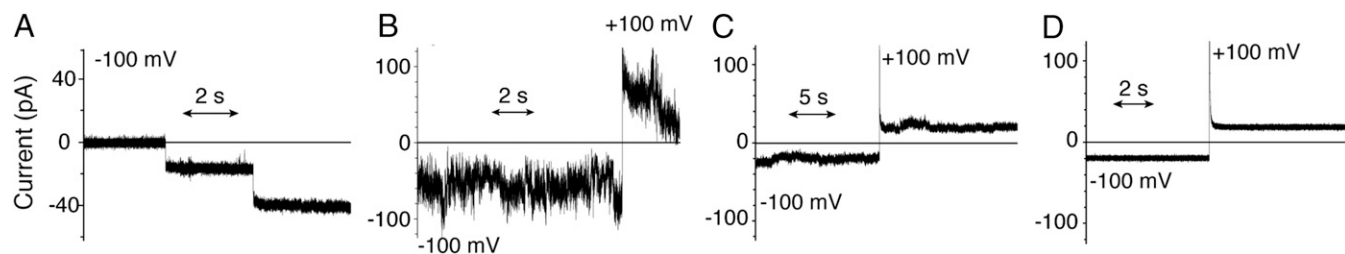


Fig. 2. A β 42 oligomers incorporate into lipid bilayers as well-defined pores. A β 42 oligomers (150- μM A β 42 concentration) were prepared under low DPC micelles at pH 7.4 and incubated for 24 h at 37 $^{\circ}\text{C}$ (referred to as $\beta\text{PFO}_{\text{A}\beta 42}$). (A) Multiple $\beta\text{PFO}_{\text{A}\beta 42}$ pore insertions. Typical current traces for type 1 (B), type 2 (C), and type 3 (D) $\beta\text{PFO}_{\text{A}\beta 42}$ pores. Electrical recordings were carried out on diphytanoyl-*sn*-glycero-3-phosphocholine planar lipid bilayers at the indicated applied potentials.

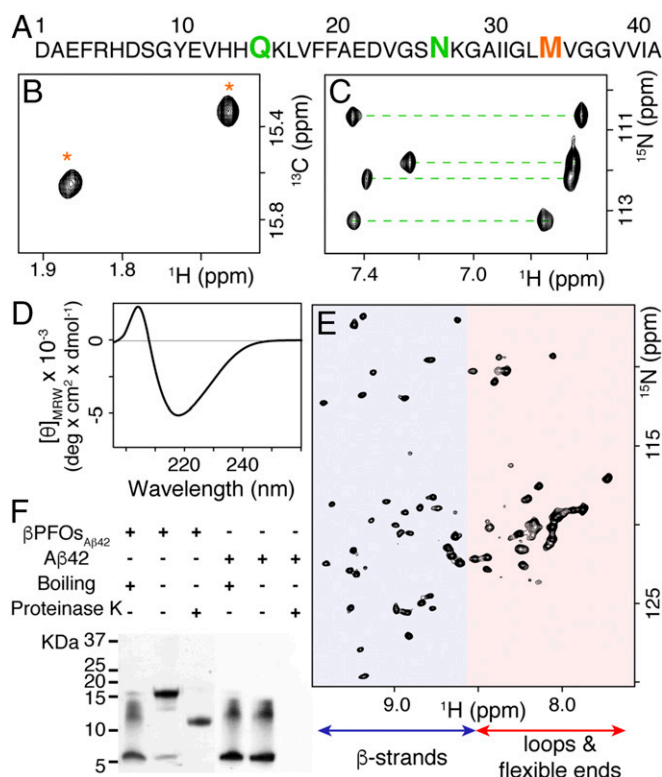


Fig. 3. Pore-forming A $\beta 42$ oligomers adopt a specific β -sheet structure with characteristics of a β -barrel arrangement. A $\beta 42$ oligomers were prepared under low DPC micelles at pH 9.0 and incubated for 24 h at 37 °C (referred to as $\beta\text{PFO}_{\text{A}\beta 42}$). (A) A $\beta 42$ sequence highlighting residues Gln-15, Asn-27, and Met-35. (B) ^1H - ^{13}C HMQC NMR spectrum of Met-35- $^{13}\text{CH}_3$ $\beta\text{PFO}_{\text{A}\beta 42}$ (1.2-mM A $\beta 42$ concentration). (C) Region of ^1H - ^{15}N HSQC NMR spectra characteristic of the side chain amides of Gln and Asn residues measured on a ^2H , ^{15}N $\beta\text{PFO}_{\text{A}\beta 42}$ sample (1.2 mM A $\beta 42$ concentration). (D) Far-UV CD characterization of $\beta\text{PFO}_{\text{A}\beta 42}$ (150- μM A $\beta 42$ concentration). (E) ^1H - ^{15}N TROSY-HSQC NMR spectrum of ^2H , ^{15}N $\beta\text{PFO}_{\text{A}\beta 42}$ (1.2-mM A $\beta 42$ concentration). The peaks clustered in the random coil region (region shown in red) would be attributable to the loops and flexible ends, whereas the downfield-shifted resonances would correspond to the β -strands of the β -barrel (region shown in blue). (F) SDS/PAGE analysis of monomeric A $\beta 42$ and $\beta\text{PFO}_{\text{A}\beta 42}$ samples with and without boiling them and before and after incubation with proteinase K.

readily exchanged with solvent deuterons, whereas those in the downfield-shifted region were resistant to exchange (SI Appendix, Fig. S6). All together, these results indicated that the structure of A $\beta 42$ oligomers comprise flexible/disordered regions, and β -strands. Distinct structural arrangements could give rise to the ^1H - ^{15}N TROSY-HSQC and CD spectra observed. However, given that A $\beta 42$ oligomers form well-defined pores in lipid bilayers (Fig. 2), the most likely structural arrangement of these oligomers is that of a β -barrel structure. In this context, the set of sharp peaks clustered in the random coil region of the spectrum would be attributable to the loops and flexible ends of the β -barrel, whereas the set of downfield-shifted resonances would correspond to the β -strands of the β -barrel (Fig. 3E) (18, 23).

To find additional evidence for a β -barrel arrangement, we analyzed A $\beta 42$ oligomers by means of experiments used to characterize membrane proteins that adopt such a structure. First, even before the first β -barrel membrane protein structure was solved, SDS/PAGE analysis revealed that β -barrel membrane proteins share a characteristic, namely that, when SDS is added, nonboiled samples retain their structure whereas boiled ones lose it (24, 25). Consequently, SDS/PAGE analysis of the nonboiled A $\beta 42$ oligomer sample led to a major band at 18 kDa, which was

assigned to the folded structure of the oligomer (Fig. 3F). Upon boiling the sample, the 18-kDa band became a 5-kDa band and a smear ranging from 11 to 14 kDa appeared, an SDS/PAGE pattern characteristic of monomeric A $\beta 42$, thereby indicating that the oligomer had been disrupted (Fig. 3F). Second, another characteristic of β -barrel membrane proteins is that proteases generate polypeptide fragments within the solvent-accessible flexible regions while leaving the β -barrel intact (23). Consistent with this finding, SDS/PAGE analysis of the nonboiled A $\beta 42$ oligomer sample previously incubated with proteinase K revealed a band at 11 kDa (Fig. 3F). To further confirm the effect of the proteases on the structure of the oligomer at atomic level, we measured a ^1H - ^{15}N HSQC spectrum of an oligomer sample prepared with ^{15}N A $\beta 42$ and incubated with proteinase K. The spectrum revealed that the resonances assigned to residues comprising loops and flexible ends shifted ^{15}N downfield and ^1H upfield within the fingerprint region, consistent with the generation of small peptides. In contrast, resonances assigned to residues comprising the β -strands remained intact (SI Appendix, Fig. S7). Taken together, the structural features of the oligomer (Fig. 3) and their pore-forming capacity (Fig. 2) are consistent with a β -barrel structural arrangement. On the basis of these findings, we named this oligomer preparation $\beta\text{PFO}_{\text{A}\beta 42}$.

$\beta\text{PFO}_{\text{A}\beta 42}$ Structural Integrity Is Maintained in Lipid Environments.

To establish that the structure of $\beta\text{PFO}_{\text{A}\beta 42}$ formed in micelles is maintained when incorporated into lipids, we reconstituted the A $\beta 42$ oligomer into bicelles. Because $\beta\text{PFO}_{\text{A}\beta 42}$ form under DPC, reconstitution of the oligomers into 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)/DPC bicelles would be extremely advantageous because it would not require additional detergent exchange steps. However, the DMPC/DPC bicelle system had only previously established for the preparation of large bicelles useful for solid-state NMR measurements (26). Therefore, we first confirmed formation of isotropic small DMPC/DPC bicelles, with molar ratio $q = [\text{DMPC}]:[\text{DPC}] = 0.33$, appropriate for solution NMR studies (SI Appendix, Fig. S8). Afterward, we reconstituted $\beta\text{PFO}_{\text{A}\beta 42}$ in these bicelles. To confirm oligomer incorporation, we performed 1D ^{15}N , ^1H -TROSY for rotational correlation times (TRACT) experiments (27) on $\beta\text{PFO}_{\text{A}\beta 42}$ samples reconstituted in DPC micelles and DMPC/DPC bicelles (SI Appendix, Fig. S9). The overall correlation time (τ_c) of $\beta\text{PFO}_{\text{A}\beta 42}$ at 37 °C was 22.4 and 30.5 ns, respectively. These values are consistent with previously reported τ_c for OmpX, an 18-kDa β -barrel protein reconstituted in DHPC micelles ($\tau_c = 21$ –24 ns measured at 30 °C) (27) and DMPC/DHPC bicelles ($\tau_c = 35$ ns measured with $q = 0.5$, at 30 °C) (28) and are in complete agreement with the fact that bicelles are larger than micelles.

To assess the number of environments detected for Met-35, Asn-27, and Gln-15 side chains of the oligomer reconstituted in bicelles, we measured ^1H - ^{13}C HMQC (Fig. 4A) and ^1H - ^{15}N HSQC (Fig. 4B) on appropriately labeled $\beta\text{PFO}_{\text{A}\beta 42}$ -bicelle samples. Spectra (Fig. 4A and B) revealed the same number of peaks as those detected in spectra for the micelle samples (Fig. 3B and C), thereby indicating that the A $\beta 42$ subunits comprising the oligomer reconstituted in bicelles also perceived two environments. Next, we measured a ^1H - ^{15}N TROSY-HSQC spectrum of ^2H , ^{15}N $\beta\text{PFO}_{\text{A}\beta 42}$. As expected because of the larger τ_c measured for $\beta\text{PFO}_{\text{A}\beta 42}$ reconstituted in bicelles, the peaks were broader (Fig. 4C) than those detected for the micelle sample (Fig. 3E). However, despite peak broadening, the bicelle spectrum showed good chemical shift dispersion and exhibited approximately the same number of peaks as their respective spectrum counterpart measured in micelles (compare Fig. 3E to Fig. 4C). Indeed, most peaks assigned to the β -strands of the β -barrel in the micelle sample were also detected, with similar chemical shifts, in the bicelle spectrum. In addition, limited proteolysis experiments carried out on the $\beta\text{PFO}_{\text{A}\beta 42}$ sample reconstituted in bicelles and analyzed by

(AFM) imaging (11). They reported donut-shaped structures and oligomeric walls protruding 1 nm above the embedding lipid bilayer surface. The donut shape and the protrusion would be consistent, respectively, with the capacity of β PFO_{sA β 42} to form pores in lipid bilayers and the presence of flexible/disordered regions outside the micelle within the β PFO_{sA β 42} structure. Using this AFM imaging data, Nussinov and coworkers carried out molecular dynamics simulations to derive atomic models for the structure of β -barrel pore-forming A β oligomers (33). This work led to pore structures with inner pore diameters slightly larger (1.7–2.5 nm) than that estimated for β PFO_{sA β 42} (0.7 nm). These models were derived by using shorter A β sequences, ranging from residues 9 to 42 or from 17 to 42, and assuming, without any direct 3D structural data, that each A β subunit within the β -barrel adopts the same structure as that of A β in the fibril. This assumption leads to a β -barrel formed by double β -sheets (33) instead of a single circular β -sheet, as described for transmembrane β -barrel proteins (18, 23). The properties of β PFO_{sA β 42} are such that they are amenable to studies designed to obtain their 3D atomic structure, thus providing a unique opportunity to obtain a 3D structure of a pore-forming A β oligomer.

Pore formation can lead to membrane leakage, which can ultimately cause a depletion of cellular energy stores, neuronal dysfunction, and neuronal death. The amyloid pore hypothesis was proposed for A β more than two decades ago (8, 9). Although there is significant evidence supporting this hypothesis, the diversity of A β pores reported (10–12) and the lack of specific structural properties characterizing them have impeded confirmation or rejection of this hypothesis. β PFO_{sA β 42} adopt a specific structure and form well-defined pores, thereby offering a unique

opportunity to establish the relevance of pore formation in the context of AD, for example by producing antibodies that specifically recognize this form of A β . Such antibodies could then be used to validate β PFO_{sA β 42} structures in relevant AD models. In addition, because, as mentioned, the properties of β PFO_{sA β 42} are amenable to 3D atomic structure studies, upon β PFO_{sA β 42} validation, the 3D structure could be used to develop new therapeutic agents. Therefore, the β PFO_{sA β 42} species whose formation and functional characteristics are described in this paper have the potential to become viable targets through which to search for new types of molecular agents designed to fight AD.

Materials and Methods

SI Appendix, Materials and Methods provides detailed protocols for the following: preparation of monomeric A β , reconstitution of A β in different detergent micelles, and preparation of A β 40 monomeric in the presence of SDS micelles and of β PFO_{sA β 42}. It also provides a description of how the electrical recordings with planar lipid bilayers and NMR studies were carried out, as well as how SEC, TEM, CD, SDS/PAGE, and limited proteolysis experiments were performed.

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